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REVERSAL OF ZOLPIDEM INTOXICATION BY SUBLINGUAL FLUMAZENIL

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14. ABSTRACT
In military operational environments, fatigue induced performance decrements resulting from reduced sleep and disrupted daily rhythms may result in outcomes ranging from severe discomfort, to mission degradation, to loss of life. In the operational environment, sleep aids cannot be used to rest the warfighter because the warfighter may be called upon to act on short notice and the sleep aid might itself degrade performance resulting in the same poor outcome. However, if the sleepiness and cognitive degradation caused by the sleep aid could be reversed, the use of sleep aids could be expanded to include some operational environments. The objective of this study was to evaluate the ability of flumazenil, administered as a liquid sublingually, to quickly and safely counteract the sleep-inducing effect of zolpidem. Thirteen participants received 10 mg zolpidem or placebo, instructed to sleep 90 minutes, and were then awakened and given either 1 mg flumazenil or placebo and tested. At the beginning of the second hour after awakening, participants were given a second sublingual; 1 mg does of flumazenil or placebo and tested over the next 5 hours. The repeated-measures, double-blind design showed that flumazenil provided partial recovery from the soporific effects of zolpidem. Conclusions: 1.Sublingual flumazenil, administered immediately on awakening, was shown to reverse the cognitively degrading effects of zolpidem by 23%, restoring performance to 92.5% of placebo. 2. One to two hours after awakening, performance did not return to the level of the placebo after flumazenil administration, but rather joined the zolpidem-only decay function which continued to be approximately 20% degraded compared to placebo. 3. At five hours post awakening, performance remained degraded by 10-11% compared to placebo. 4. Consideration should be given to developing a new means of administering flumazenil in a form that can be self-administered and quickly dissolved when placed under the tongue.

15. SUBJECT TERMS
Flumazenil, Zolpidem, Sublingual administration, Fatigue management, Cognitive performance, Sleep

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PREFACE

The contract technical monitor for this work was Dr. James C. Miller and the program managers were Lt Andrea Pinchak and Lt Andrew Workman of the Biosciences and Protection Division. The work was performed under work unit number CRDAP105.

This research was sponsored, funded, and approved by the U.S. Army Medical Research and Materiel Command under Contract Award No. DAMD17-00-2-0055 to NTI, Inc. No financial or other relationships existed that might be perceived as leading to a conflict of interest. The study was conducted using Air Force Research Laboratory facilities at Brooks City-Base, TX, under the Cooperative Research and Development Agreement 01-225-HE-01 between NTI, Inc, and the Biosciences and Protection Division (AFRL/RHP). Cory Welch, Amy McCrory, Juan Mendez, and Heather Campbell provided outstanding technical assistance. We thank Roel Arrendondo, Wilford Hall Medical Center, for preparation and management of the drugs and Mr. Joe Fischer, General Dynamics, for drug randomization and statistical analysis. The views expressed in this paper are those of the authors and do not reflect the official position of the Department of the Air Force, Department of the Army, Department of Defense, or the U.S. Government.

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INTRODUCTION

Fatigue resulting from reduced sleep and disrupted circadian rhythms (daily sleep/wake cycles) is well established to cause significant decrements in cognitive or mental performance. In military operational environments, fatigue induced performance decrements resulting from reduced sleep and disrupted daily rhythms may result in outcomes ranging from severe discomfort, to mission degradation, to loss of life. Commonly used fatigue countermeasures such as improved sleeping conditions and more frequent rest breaks are sometimes insufficient or are not available options to counter the effects of the cumulative fatigue caused by disrupted and lost sleep during extreme sustained and long-duration military operations. In these critical situations, military commanders and physicians may jointly approve the controlled and limited operational application of sleep-aid medications to promote and enhance sleep during opportunities for rest and recovery under less than ideal sleeping conditions. These sleep medications have been previously approved by the Food and Drug Administration (FDA) for routine use to induce and maintain sleep in adults with various sleep disorders. The considerable advantages of using selected sleep-aid drugs to enhance sleep and subsequent performance in military personnel participating in sustained operations has been well documented in a number of recent conflicts, including Operation Desert Storm and Operation Iraqi Freedom (Cornum, Cornum, & Storm, 1995; Emonson & Vanderbeek, 1995). However, because the very reason for using these drugs is that they promote drowsiness and sleep, personnel administered a sleep-aid to enhance rest may not be able to remain alert and perform effectively if awakened prematurely while under the drug's influence. Thus, for military operations, it would be very useful to have available, when needed, another drug that could be readily self-administered to counteract the sleepiness effects of a recently administered sleep-aid drug.

Zolpidem tartrate (Ambien®, Sanofi-Aventis) is one of three hypnotic compounds approved by the USAF Surgeon General for use to promote sleep in aircrews and special duty personnel that must acquire pre-mission crew rest under adverse and demanding operational situations (the two other USAF approved sleep-aids are temazepam and zaleplon). Prior to reporting for airborne missions USAF aircrews are required by regulation to receive 12 hours of inviolate crew rest during which they must be afforded the opportunity for at least eight hours of uninterrupted sleep. When approved for use by the unit commander and flight surgeon the recommended therapeutic dose of 10 mg zolpidem may be taken no less than six hours before reporting for the scheduled crew duty day and mission. Zolpidem's pharmacokinetic profile makes its designated application during the regulated 12-hour aircrew rest periods effective and safe. Peak plasma concentrations are reached 1.0-1.5 hours after ingestion and the elimination half-life is 2.0-2.5 hours.

Decisions on the use of zolpidem to enhance the restorative value of sleep during crew-rest must weigh the benefits and risks given the nature of the military operation, the condition of the personnel, the sleeping environment, and the likelihood that the sleep could be interrupted while under the influence of zolpidem. Studies seldom find residual effects following an uninterrupted night's sleep or extended daytime sleep with 10 or 20 mg zolpidem (Caldwell, Prazinko, Rowe, et al., 2003; Eddy, Barton, Cardenas, et al., 2006). However, emergency and contingency situations can arise during intense, sustained military operations that require sleeping personnel be awakened prior to completion of their allotted sleep period. The sedation

induced by zolpidem is the result of central nervous system depression and personnel may be ineffective until the soporific effects of the compound wear off (Storm, Eddy, Welch, et al., 2007). Cognitive performance and alertness have consistently been found to be impaired when zolpidem is present at peak or near-peak plasma levels during the hours subsequent to ingestion. Thus, for these emergency and contingency situations, military operations could benefit from an agent that would counteract the soporific effects of a sleep-aid drug.

Flumazenil (Romazicon®) is an imidazobenzodiazepine derivative approved by the Food and Drug Administration (FDA) to be given intravenously in clinical settings. It antagonizes the actions of benzodiazepines on the central nervous system by competitively inhibiting activity at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex. Flumazenil is a weak partial agonist in some animal models of its pharmacologic activity, but has little or no agonist activity in man. Flumazenil does not antagonize the central nervous system effects of drugs affecting GABA-ergic neurons by means other than the benzodiazepine receptor (including ethanol, barbiturates, or general anesthetics) and does not reverse the effects of opioids (Romazicon® package insert). Flumazenil does not appear to change zolpidem plasma concentrations, suggesting a pharmacodynamic interaction (Patat, Naef, van Gessel, et al., 1994). The manufacturer, Roche, notes, "The pharmacokinetics of benzodiazepines is unaltered in the presence of flumazenil."

When administered immediately after surgeries flumazenil shortens the time required for recovery from the sedative effects of surgical anesthetics. It also reverses the effects of overdoses of sleep-aid drugs including zolpidem. Flumazenil has been used to antagonize sedation, impairment of recall, psychomotor impairment, and ventilatory depression produced by overdoses of benzodiazepines. Wesensten, Balkin, Davis, et al., (1995) administered 20 mg zolpidem or 0.5 mg triazolam immediately followed by 90 minutes of daytime sleep. Intravenous flumazenil administered immediately on awakening prevented both immediate and delayed memory impairment by either drug, although sedation effects returned six hours after zolpidem administration.

Currently the only effective method of administering flumazenil is through intravenous administration. Obviously, intravenous administration of flumazenil under the conditions of an operational emergency or sudden call-to-duty is militarily impractical. The present study evaluated the efficacy of sublingual doses of flumazenil to counteract the soporific effects of zolpidem on cognitive performance in an operationally-relevant, sudden-awakening paradigm.

FLUMAZENIL ELIMINATION

Metabolism

Flumazenil has an elimination half-life of 54 minutes (range 41 – 79), and is primarily metabolized by the liver forming two inactive metabolites that are excreted in the urine. It is primarily hydrolyzed by a liver carboxylesterase to flumazenil acid and N-demethylated flumazenil, probably by the cytochrome P-450, as are other benzodiazepine compounds (Kleingeist, Böcker, Geisslinger & Brugger, 1998). This remains to be determined.

Competition with Benzodiazepines

Binding of benzodiazepines to the gamma-aminobutyric acid receptor occurs at the ω_1 and ω_2 subunits. Flumazenil does not discriminate between the subunits and has a dissociation coefficient of 0.60 ng/L (Lowenstein, Rosenstein, Caputti & Cardinali, 1984). Flumazenil is approximately 50% bound to serum protein (Romazicon® package insert). Zolpidem, an imidazopyridine, is highly selective for the ω_1 subunit, and has a similar dissociation coefficient of 1.5 – 2.1 ng/L (Munakata, Jin, Akaike, & Nielsen, 1998). Several studies have examined the pharmacokinetic interaction of flumazenil with hypnotic agents. Patat, Naef, van Gessel, et al. (1994) found that while effective for reversing zolpidem-induced sedation and psychomotor impairment, 0.04 mg/kg of intravenous flumazenil had no effect on zolpidem pharmacokinetics. This study was unusual in that zolpidem was administered intravenously rather than orally and found a mean serum elimination half-life for zolpidem of 1.2 hours versus 2.4 hours after oral dosing. One small study found that 1 mg of intravenous flumazenil prolonged the elimination half-life of 0.1 – 0.2 mg/kg of midazolam, a short-acting imidazobenzodiazepine (Bonfiglio, Fisher-Katz, Saltis, et al., 1996). A study by Rogers, Morrison, Nafziger, et al. (2002) found that a smaller dose of intravenous flumazenil, 0.005 mg/kg, reversed impairment on the Digit Symbol Substitution Test resulting from 0.025 mg/kg of midazolam without significantly altering midazolam pharmacokinetics.

It is possible that competition for elimination via the liver exists for flumazenil and hypnotic agents, such as zolpidem, but this is only seen when the quantities of both drugs are sufficient to saturate the liver CYP 3A4 enzyme binding. The zolpidem displaced from ω_1 and CYP 3A4 sites could remain in the serum or bind to another, unknown receptor.

FLUMAZENIL FORMULATION AND ADMINISTRATION

Intravenous Solution Administered Sublingually

Currently, the only FDA approved formulation of flumazenil is a solution for intravenous administration, 1-mg per 10-ml. The time and logistical requirements for intravenous administration preclude this route of administration for military operational use. Flumazenil has been administered via other routes in research and clinical trials. Flumazenil pharmacokinetics were compared for oral administration (30-mg) versus intravenous administration (2-mg) in healthy young and elderly persons (Roncari, Timm, Zumbrunnen, et al., 1993). Bioavailability was found to be about 25%. Orally administered flumazenil reduced diastolic blood pressure. Side effects described were dizziness, mild confusion, and circulatory insufficiency. Though these were considered mild, they are not compatible with military operations, particularly the aerospace environment. Nasal administration has been used to reverse sedation in pediatric anesthesia (Scheepers, Montgomery, Kinahan, et al., 2000). Submucosal administration was compared to intravenous administration in dogs (Oliver, Sweatman, Unkel, et al., 2000). One study compared flumazenil administration (0.2-mg, then another 0.3-mg 30 seconds later) for the reversal of benzodiazepine-induced respiratory depression in dogs via intravenous (IV), sublingual (SL), intramuscular (IM), and rectal (PR) routes (Heniff, Morre, Trout, et al., 1997). The rapidity of reversal (in seconds) was: IV 120 ± 24.5, SL 262 ± 94.5, IM 310 ± 133.7, and PR 342 ± 84.4. The mean difference in time between IV and SL administration, 142 seconds, is far

less than the time to establish intravenous access for administering flumazenil. This makes the SL route attractive for military operational use.

GOAL OF STUDY

The goal of the present study was to demonstrate the feasibility of delivering flumazenil by the sublingual route in humans and to determine its effects on cognitive performance, physiological performance, and side effects. It was understood that our method would not ensure 100% bioavailability. With the success of this feasibility study, it was hoped that it would stimulate the formulation and testing of a field ready product. Such a product might find use in military operations as a safe way to rest fatigued warfighters without diminishing their fighting capacity.

METHODS

PARTICIPANTS

Five women and eight men (mean age 28.8 years, range 20-42 years) completed the study. Volunteers were thoroughly briefed on the possible risks and discomforts associated with participation and medically examined (including blood chemistry and liver function) by a qualified medical practitioner knowledgeable of the objectives and requirements of the study. Volunteers with evidence of any current significant illness, sleep abnormalities, use of tobacco, excessive use of caffeine or alcohol, or being excessively over- or underweight were not allowed to participate. The medical examination assured that participants were not currently using drugs that might interact with those being evaluated in the study. Women who were pregnant or attempting to become pregnant were excluded. Female participants were administered a urine pregnancy test immediately prior to each experimental session. One subject was dropped from the analyses due to presenting with Lasik surgery two days prior to testing and having numerous visual problems. The research protocol and Informed Consent Document (ICD) were reviewed and approved by the Brooks City-Base Institutional Review Board (IRB) in advance of participant recruitment. Participants gave written informed consent before participating and were paid for their participation. Review by the FDA determined that an Investigational New Drug (IND) application was not required for the protocol.

PREPARATION OF SUBLINGUAL FLUMAZENIL

Flumazenil is insoluble in water but mostly soluble in acidic solutions. The intravenous formulation is adjusted to a pH of 4 (approximately the acidity of ascorbic acid). It has a slightly bitter and salty taste. Lemon extract was used to mask this taste by adding 1 ml of McCormick's Pure Lemon Extract (alcohol 84%, water and oil of lemon) to each 10 ml of flumazenil solution. Theoretically, this was adequate to maintain a pH of ≤ 4; the actual pH of the end solution was not assayed. The flumazenil placebo was formulated by substituting distilled water for the flumazenil solution. To facilitate sublingual administration, five aliquots of approximately 2 ml each (1 mg of flumazenil) were drawn into syringes. This allowed a comfortable volume of solution beneath the tongue. Drug and placebo packaging with freshly made solutions and blinding were performed by one investigator (JAG) and the pharmacy staff at USAF Wilford

Hall Medical Center (WHMC), Lackland AFB, San Antonio, Texas, on the morning of each experimental session. The prepared syringes were then labeled appropriately for each participant by the pharmacy staff, ensuring that all investigators remained blind to what medications each participant received.

FACILITY AND MATERIALS

This study was conducted at the Air Force Research Laboratory Biosciences and Protection Division (AFRL/RHP), Fatigue Countermeasures Lab (FCL) located at Brooks City-Base, Texas. During the experimental sessions each participant was assigned to a private room equipped with a computer and desk for testing, a bed, an easy chair, and a private bath. Throughout the experimental sessions the participants were always under the direct observation of research personnel or knowingly monitored from a central control station by closed circuit television, excluding of course the private baths. Infra-red capability allowed monitoring of the participants while sleeping in the darkened rooms. An intercom system allowed the participants to contact the investigators at any time.

Controlled drugs were managed in accordance with AFRL/RHP Operating Instruction 44-102, "Research Drug Control." Facilities within AFRL/RHP and the FCL comply with Drug Enforcement Agency (DEA) and USAF requirements for the storage and maintenance of FDA Schedule II-V pharmaceuticals. Two of the investigators (DRE and JAG) were registered with the DEA and the Texas Department of Public Safety and certified to dispense for study Schedule II-V drugs. Zolpidem 10 and 20 mg tablets were obtained from the WHMC pharmacy's normal stock and packed in standard size gelatin capsules using orange-flavored psyllium as filler. The placebo sleep aid consisted of the gelatin capsule filled with orange-flavored psyllium. The flumazenil used in the study was provided by the WHMC pharmacy, 10 ml multiple-use vials containing 0.1 mg/ml.

The FDA recommends that flumazenil be administered as a distributed series of small injections for the reversal of the sedative effects of benzodiazepines administered for conscious sedation. The recommended initial dose of flumazenil is 0.2 mg (2 ml) administered intravenously over 15 seconds. If the desired level of consciousness is not obtained after waiting an additional 45 seconds, a further dose of 0.2 mg (2 ml) can be injected and repeated at 60-second intervals where necessary to a maximum total dose of 1 mg (10 ml). In the event of re-sedation, repeated doses may be administered at 20-minute intervals as needed. For repeat treatments no more than 1 mg, given as 0.2 mg/min, should be administered at any one time, and no more than 3 mg should be given in any one hour. Considering the FDA guidance, sublingual doses of flumazenil for this study were administered using small, blunt syringes filled with 2 ml of solution, flumazenil or placebo. A 1 mg dose of flumazenil or placebo consisted of administering five syringes at one-minute intervals.

EXPERIMENTAL DESIGN

This study employed a double-blind, repeated-measures design. Four combinations of sleep-aid/sleep-aid-countermeasure treatments were evaluated (see Table 1): passive control (zolpidem-placebo + flumazenil-placebo; "P/P"); zolpidem active control (10 mg zolpidem +

flumazenil-placebo; "Z10/P"); experimental condition 1 (10 mg zolpidem + 2, 1 mg flumazenil; "Z10/F"); experimental condition 2 (20 mg zolpidem + 2, 1 mg flumazenil; "Z20/F"). A zolpidem-placebo + flumazenil condition was not included since it has been demonstrated that flumazenil has no intrinsic alerting effects on performance when administered alone (Wesensten, et al., 1995). A 20 mg zolpidem + flumazenil-placebo condition was not included since, compared to the Z10/P condition, sedative effects would merely be lengthened.

Table 1. Treatment Conditions for Flumazenil Study.

Condition	Sleep Aid	Countermeasure	Abbreviation in Text and Figures
Passive Control	Placebo	Placebo	P/P
Zolpidem Active Control	Zolpidem 10 mg	Placebo	Z10/P
Experimental Condition 1	Zolpidem 10 mg	Flumazenil 2, 1 mg	Z10/F
Experimental Condition 2	Zolpidem 20 mg	Flumazenil 2, 1 mg	Z20/F

Four groups, each comprised of 2-4 participants, were randomly assigned to different 4x4 Latin squares, with each participant exposed to a different drug treatment during each of his/her four experimental sessions. The first (n=4) and second (n=4) groups completed their four experimental sessions during the same four consecutive weeks, one session per week. The third (n=3) and fourth (n=2) groups were subsequently tested on a similar four-week schedule. At the point of the first and second groups having completed data collection for their first two experimental sessions, the medical monitor determined that the Z20/F condition had, in four of four cases, resulted in considerable nausea and/or emesis on or soon after awakening and for up to five hours post-awakening, with there being no apparent relief from flumazenil. The medical monitor, the investigators, and the IRB considered it inappropriate and unnecessary to continue the Z20/F condition in the study. Unbeknown to the participants, but with expeditious review and approval of the IRB so as not to delay the testing schedule, the Z20/F condition was replaced for the remainder of the study by a second administration of the P/P condition. This modification maintained the experimental design and testing milieu and allowed data collection to be completed for the Z10/F, Z10/P, and P/P conditions. The first and second groups were informed of the modification following completion of their fourth experimental session. Prior to initiating data collection for the third and fourth groups the IRB approved a modified protocol and ICD incorporating the deletion of the Z20/F condition to which the participants gave updated written informed consent. The limited and incomplete data collected under the Z20/F condition were not included in the statistical analyses.

TESTS AND MEASURES

Automated Neuropsychological Assessment Metrics (ANAM)

Four cognitive performance assessment tasks from the PC-based ANAM battery (Reeves, Winter, Kane, et al., 2001) were applied in this study. The four tasks required a total of about 14 minutes for a well-practiced, alert participant to complete under baseline conditions. Response times and correct and incorrect responses were recorded. The four ANAM tasks were performed in the following sequence during each testing block.

1. *Reaction Time* – Simple Reaction Time – a participant pressed a computer mouse key in response to a visual stimulus presented at a centrally fixed point on the computer screen. The time from stimulus onset to key press was recorded; the outcome measure was mean reaction time to 20 stimuli (inter-stimulus interval of 650-1200 msec); trial duration was less than one-minute.
2. *Mathematical Processing* – Each problem in this task included two mathematical operations (addition and/or subtraction) on sets of three single-digit numbers (e.g., 5+3–4=?). The participant was instructed to read and calculate from left to right and indicate whether the sum was greater-than '5' with a right mouse key press or less-than '5' with a left mouse key press. Trials were three minutes in duration.
3. *Grammatical Reasoning* – The participant determined as quickly as possible whether two simple summary statements (e.g., *& follows** and *# precedes**) correctly described the sequential relationship among three symbols (e.g., *# &**). If one statement was true and one false, the right mouse key was pressed; if both statements were true or both were false the left mouse key was pressed. A trial consisted of 48 presentations.
4. *Continuous Processing* – Participants were directed to continuously monitor a randomized sequence of the numerals 0 through 9 presented one at a time in the center of the screen and to press the left mouse key if the numeral currently on the screen matched the numeral that immediately preceded it. If not a match, they were to press the right mouse key. Trials were three minutes in duration. (This task is also referred to as Running Memory.)

Williams Word Memory Task

The Williams Word Memory Task provided an assessment of short-term memory. During the first post-awakening test block at 1500, the participant listened to an auditory presentation of 15 recorded words. Each word was spoken, spelled, and then spoken again. The participant wrote down each word as it was presented. On completion of the presentation, the participant studied the list for one minute. The written list was then collected and the participant was directed to immediately recall in one minute as many of the words as possible by writing them on a blank sheet of paper. Delayed recall of the same list occurred two hours later during the third post-awakening test block at 1700. The number of words recalled from the list of 15 was the outcome measure for this task.

Psychomotor Vigilance Task (PVT)

The PVT (Model PVT-192, CWE Inc., Ardmore, PA) is a hand-held, self-contained visual reaction time task requiring sustained attention and a simple, discrete key press to each signal - the onset of an elapsed-time digital clock. The device was enclosed in a plastic case that measured 21 X 11 X 6 cm and weighed 658 g. The clock appeared within a well-defined display window and was extinguished and reset to zero within a second after each response. Signals occurred randomly every 2-12 seconds. Trials were 10 minutes in duration and the outcome measure was mean reaction time and mean reciprocal reaction time.

Postural Sway

Postural or body sway was assessed using a force platform that measured changes in the body's center of pressure over time (Platform model OR6-5-1, AMTI, Watertown, MA). The apparatus resembles an oversized home bathroom scale, approximately 45.7 by 50.8 cm in area and 7.6 cm in height. The participant was directed to stand as motionless as possible while one minute of data was collected for both eyes open and eyes closed conditions at a sampling rate of 10 Hz. The amplitude, velocity, and frequency of change in the center of pressure measured the participant's ability to maintain balance. An elliptical area of measurement that accounts for 95% of the variation in the center of changes in pressure provided the outcome measure.

Grip Strength

Strength was measured as the highest value attained of two grip squeezes, separated by one minute, on a Sammons-Preston, Inc. JAMAR (Bolingbrook, IL) hydraulic hand dynamometer.

Sleepiness

The ANAM battery offers a sleepiness scale that, while a modification of the Stanford Sleepiness Scale (Hoddes, Zarcone, Smythe, et al., 1973), maintains the seven-point scale rating subjective sleepiness from "*1 - very alert, wide awake, and energetic*" to "*7 - very sleepy and cannot stay awake much longer*." The ANAM sleepiness scale was presented on the computer monitor at the start of each testing block and the participant was instructed to press the numerical key corresponding to their immediate feeling of sleepiness.

Symptoms

Participants completed a 73-item paper and pencil Symptom Checklist at the end of each test block, indicating the severity (*none, some, moderately, or severely*) they were experiencing for each symptom at that point in time

Activity Log

Each participant was provided with a formatted log to manually record his/her wake and sleep times daily throughout training and recovery.

PROCEDURES

During selection and training the participants were given considerable orientation on the study objectives and the relevance of the experimental manipulations to real-world operations. The importance of maintaining standardized procedures and performing the cognitive tasks as rapidly and accurately as possible was emphasized. Prior to their initial experimental session participants were trained to asymptotic performance on each of the cognitive tasks and became proficient on the procedures for transitioning efficiently from one task or procedure to the next. Coaching and practice were also provided on self-administration of solutions using the blunt

needle syringes until each participant was comfortable with the sublingual procedure. Using water for training, participants were taught to empty a syringe into their buccal cavity in 10-15 seconds and to hold the fluid in their mouth for 45-50 seconds as timed by an attending research observer. At the end of the timed interval, the participant was directed to swallow the remnant fluid and immediately self-administer the next of the five syringes to simulate the administration of a complete single dose.

The testing schedule for the experimental sessions is presented in Table 2 starting with Test Session 1. The participants were directed to sleep from about 10:00 pm to 7:00 am the night before scheduled experimental sessions, and to not consume alcoholic beverages the evening prior to or the day of a session. Experimental sessions began at 1200 and were completed at 2100. Participants were allowed time to settle into their rooms and have a light lunch prior to the baseline testing block at 1230. During each session the participants completed one test block before and six blocks after a 1.5-hour sleep period. Each test block was about 50 minutes in duration, with the balance of the hour serving as a brief rest-break. Except for including the Williams Word Memory Task in the 1500 and 1700 blocks, all seven testing blocks were identical. The sleep aid was administered at 1330, participants were encouraged to sleep and the lights were extinguished.

Table 2. Test Session 1 – Experimental Treatment

Session	Time	Procedure
Session 1	1130-1200	Arrive FCL; Attach instruments; Collect logs/actigraphs
	1200-1230	*Light lunch/break*
	1230-1300	ANAM, PVT, Surveys
	1300-1315	FP/GS/Vitals*
	1315-1330	*Break*
	1330-1500	**Sleep Aid Dose** / Sleep (lights out)
	1500-1530	*Countermeasure* Dose/ANAM+WMm, Surveys
	1530-1555	PVT, FP/GS/Vitals*
	1555-1600	Break
	1600-1630	*Countermeasure* Dose/ANAM, Surveys
	1630-1655	PVT, FP/GS/Vitals*
	1655-1700	Break
	1700-1720	ANAM, surveys
	1720-1745	PVT, FP/GS/Vitals*
	1745-1800	*Break/Snack*/Detach instruments
	1800-1820	ANAM, Surveys
	1820-1845	PVT, FP/GS/Vitals*
	1845-1900	*Break*
	1900-1930	ANAM+WMr, Surveys
	1930-1955	PVT, FP/GS/Vitals*
	1955-2000	*Break*
	2000-2020	ANAM, Surveys
	2020-2045	PVT, FP/GS/Vitals*
	2045-2100	Hand out logs/actigraphs; release
(*FP:Force Platform; GP:Grip Strength; Vitals: BP, HR, temperature)		
Session 2	Same as Session 1 – Dose 2	
Session 3	Same as Session 1 – Dose 3	
Session 4	Same as Session 1 – Dose 4	

The drug doses were always ingested under the close observation and attendance of an investigator or senior technician. Sleep-aid capsules (zolpidem or placebo) were orally ingested within 2-3 minutes of 1330, following which the participants were immediately shepherded to bed, the room door closed, and the lights turned off. Participants were instructed to remain in bed for the 1.5-hour duration even if they could not fall or remain asleep. The participants were awakened at 1500 by voice instruction over the intercom system and simultaneously lights were illuminated, followed immediately by research staff entering each room to monitor the participants in the administration of the flumazenil treatment. The FDA-approved, distributed-dose-schedule for administering zolpidem intravenously as a countermeasure to the sedative effects of the zolpidem was employed. Two, 1 mg sublingual doses of flumazenil were administered one hour apart to counteract the sedative effects of the zolpidem ingested 1.5 hours prior to the first countermeasure dose. On being awakened, the participants were assisted as required to a comfortable sitting position in bed. They then self-administered sublingually, at one-minute intervals, the five syringes (2 ml each) comprising the total 1 mg countermeasure or placebo dose. The participants were then assisted the few steps to their computer station where they performed the 1500 test block. A second 1 mg dose was administered one hour later at 1600 just before the 1600 test block using the same method. In this case the participant self-administered the five syringes sublingually while sitting at his/her computer testing station.

Vital signs (blood pressure, heart rate, and oral temperature) were monitored once during each testing block. Water and selected non-caffeinated drinks were available throughout the experimental sessions. Lying down or sleeping were never permitted except during the scheduled sleep period integral to the study. Participants were required to make arrangements to be chauffeured home from the laboratory on completion of each testing session. Once home, participants were directed to acquire at least six hours of sleep prior to operating machinery, driving, or performing similar tasks that may involve hazards.

STATISTICAL ANALYSES

Before statistical analyses were performed, the data for each outcome measure was baseline adjusted (with the exception of the Word Memory test, which has no baseline) to compensate for any week to week variation that might have occurred within a participant. This was accomplished for each experimental session by subtracting a participant's baseline value (i.e., pre sleep time point) from the value at each data collection time point. This change-from-baseline-data was used as the "raw" data for statistical analysis.

For each continuous, normally distributed, outcome measure, a repeated-measures analysis of variance with two within-subject factors: drug condition (the three drug combinations) and time (the six post-awakening data collection periods) was performed on the change data to test for significant drug and time main effects and/or a drug by time interaction. When significant drug effects were detected, post-hoc simple effects tests (Winer, 1971, p. 174) were used to compare the drug conditions at each time, separately. For discrete outcome measures, and measures where non-normality was suspected, non-parametric procedures (Friedman's test and Wilcoxon signed-rank test) were performed to compare the drug conditions at each time, separately. The Statistical Package for the Social Sciences (SPSS Version 15) was used for the computations.

We wanted to evaluate and report only operationally significant flumazenil-reversals of sleep aid intoxication, compared to the flumazenil placebo (Active Control), as being significant. Thus the effect size was set at 1 standard deviation unit (sdu). To insure sufficient power for identifying specific differences, we based our power analysis on the post-hoc simple effects tests. Therefore the study was planned with a sample size of 16, testing at an alpha level of 0.05 giving a power of 96%; however, only 12 participants completed the study. When testing at the 0.05 alpha level, a sample of 12 participants provided an 88% chance (power) of detecting a difference of 1 sdu when comparing any two drug conditions at a given time point. Since the desired flumazenil-reversal performance was expected to be no different than performance in the Passive Control condition, the power would be the same with the identical assumptions.

RESULTS

Measures of accuracy, mean reaction time, and throughput (which is a combined measure of accuracy and speed, and is defined as the number of correct responses per minute), were available for the grammatical reasoning, mathematical processing, and continuous processing tasks. Mean reaction time and mean reciprocal reaction time were analyzed for the PVT in addition to mean reaction time for the simple 20-item reaction time task. Memory, physiological, and subjective measures were also analyzed.

COGNITIVE PERFORMANCE

Grammatical Reasoning

For accuracy, no significant effects were detected by the ANOVA. Table 3 shows, for each drug condition, the baseline mean (and standard deviation) and the mean change from baseline (and standard deviation) at each post-awakening time. For mean reaction time shown in Table 4, the drug by time interaction was significant allowing pair wise comparisons of the drug conditions at each test time ($F(4, 46) = 3.066$, $p = 0.024$ using Huynh-Feldt correction). The Z10/P change was significantly larger than that for the P/P condition at 1500, 1600, and 1700 ($p < 0.05$) and approached significance at the 1900 testing session ($p = 0.053$) showing the degrading effects of this drug on reaction time. Similarly, the Z10/F change was significantly larger than that for P/P at the 1600, 1700, and 1800 testing sessions ($p < 0.05$) and the 1500 testing session approached significance ($p = 0.062$). The difference between Z10/P change and the Z10/F change approached significance at 1500 and 1600 ($p = 0.067$ and 0.051, respectively). Figure 1 shows that reaction time for Z10/F was intermediate between P/P and Z10/P for the first few hours after awakening.

Table 3. Change from Baseline in Each Drug Condition for Grammatical Reasoning Accuracy (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	96.18 (2.64)	96.01 (3.71)	97.74 (3.01)
1500	0.18 (4.12)	-10.24 (20.19)	-0.52 (3.78)
1600	0.70 (4.47)	-0.91 (6.51)	-1.93 (4.04)
1700	0.35 (4.34)	-5.76 (14.81)	-1.74 (5.88)
1800	-1.91 (8.54)	-2.60 (15.79)	-2.43 (5.17)
1900	-2.08 (8.62)	-3.42 (18.73)	-1.91 (6.73)
2000	-0.52 (6.83)	-2.79 (11.46)	-1.56 (4.87)

Table 4. Change from Baseline in Each Drug Condition for Grammatical Reasoning Mean Reaction Time (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	4102 (899)	4251 (1053)	4192 (820)
1500	105 (299)	2129 (1970)*	736 (1107)
1600	79 (460)	1455 (1400)*	884 (1000)*
1700	-259 (293)	1399 (1619)*	1048 (1557)*
1800	-87 (605)	802 (1671)	976 (1102)*
1900	-58 (478)	493 (591)	655 (1149)
2000	18 (580)	310 (475)	393 (920)
* significantly different from P/P ($p \leq 0.05$)			

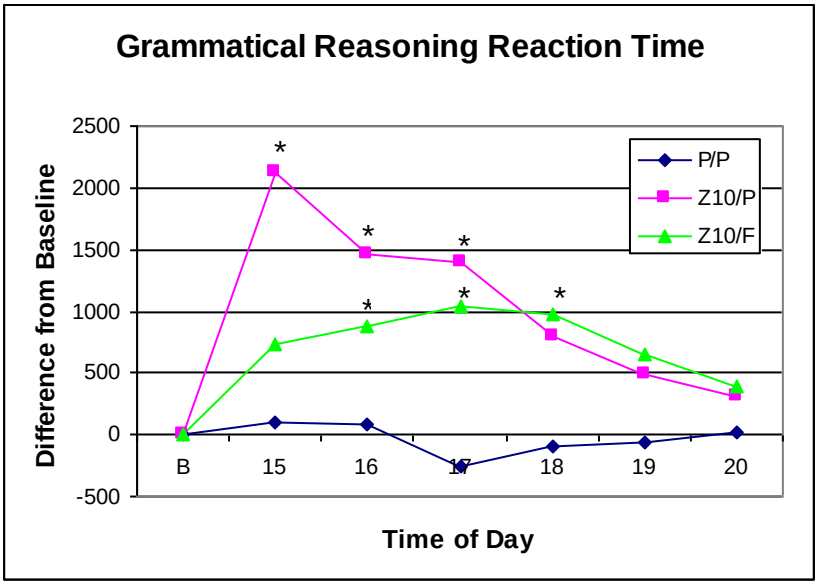

Figure 1. Grammatical reasoning reaction time as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P.)

The drug by time interaction for grammatical reasoning throughput was significant ($F(4, 53) = 2.682$, $p = .032$ using Huynh-Feldt correction) allowing pair wise comparisons of the drug conditions at each test time. Changes from baseline are shown in Table 5 for each drug condition. For pair wise comparisons the Z10/P change from baseline differed significantly from the P/P condition at 1500, 1600, 1700, and 1800 ($p < 0.05$), while the Z10/F change differed significantly from the P/P change only at 1600 and 1700 ($p < 0.05$). Z10/F approached significance at the 1800 testing session ($p = .069$). Figure 1 shows the degrading effects of zolpidem on grammatical reasoning throughput with significant recovery from flumazenil only during the first hour with little recovery thereafter. The Z10/P and Z10/F conditions did not differ from each other at any time point. The P/P condition changed from baseline by increasing 1.27 ($t(11) = 2.70$, $p = 0.021$) at 1700.

Table 5. Change from Baseline in Each Drug Condition for Grammatical Reasoning Throughput (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	14.59 (3.40)	14.30 (3.68)	14.55 (3.19)
1500	-0.01 (1.76)	-4.69 (4.78)*	-1.64 (3.02)
1600	0.11 (2.05)	-2.81 (3.49)*	-2.40 (2.33)*
1700	1.27 (1.62)	-3.64 (4.39)*	-2.89 (4.36)*
1800	0.46 (3.79)	-2.07 (3.78)*	-3.06 (3.47)
1900	0.18 (2.17)	-1.47 (2.60)	-1.88 (3.89)
2000	0.17 (1.87)	-1.20 (1.97)	-1.47 (3.15)
* significantly different from P/P ($p \leq 0.05$)			

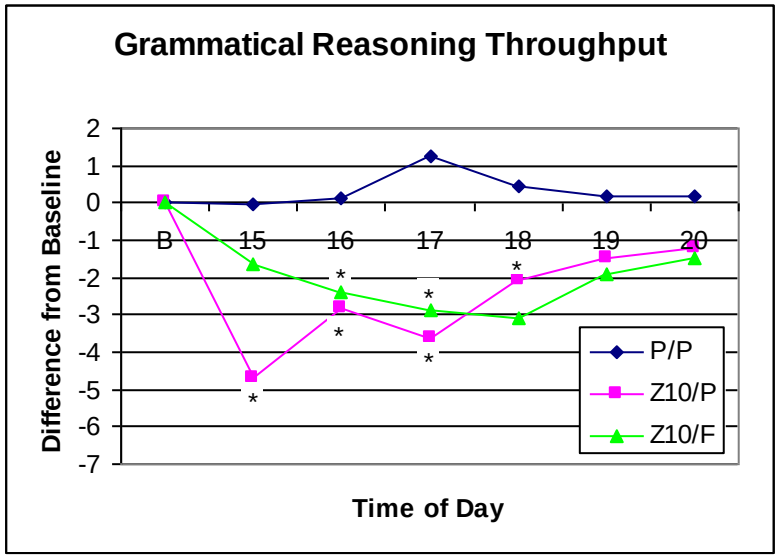

Figure 2. Grammatical reasoning throughput as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P.)

Mathematical Processing

No significant effects were detected by ANOVA for mathematical processing accuracy. The change data are shown in Table 6. For mean reaction time, the ANOVA showed significant effects for drug and time ($p < 0.05$), but not for the drug by time interaction ($F(12, 120) = 1.47$, $p = 0.144$). Significant changes from baseline were found for Z10/P and for Z10/F when compared to P/P at 1600, 1700, and 1800 ($p < 0.05$) and at 2000 for Z10/F ($p = 0.012$) as shown in Table 7. Z10/P approached significance at the 1500 testing session ($p = .071$). Figure 3 shows performance effects similar to grammatical reasoning. The lack of significance at 1500 was likely due to the joint effect of an increased RT in the P/P condition, Figure 3, and high variability in the Z10/P condition, Table 7.

Table 6. Change from Baseline in Each Drug Condition for Mathematical Processing Accuracy (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	96.47 (2.42)	98.48 (1.53)	97.21 (2.44)
1500	-1.02 (3.53)	-8.79 (9.50)	-2.29 (5.74)
1600	-0.07 (2.95)	-2.86 (8.05)	-1.40 (3.52)
1700	-0.16 (3.06)	-7.75 (22.24)	-2.24 (7.99)
1800	0.05 (2.93)	-8.46 (20.92)	-0.15 (3.59)
1900	-2.27 (6.27)	-6.38 (19.80)	-0.54 (3.91)
2000	-3.48 (6.26)	-4.33 (11.69)	-1.04 (7.53)

Table 7. Change from Baseline in Each Drug Condition for Mathematical Processing Mean Reaction Time (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	1566 (584)	1558 (545)	1520 (455)
1500	114 (234)	490 (649)	235 (340)
1600	40 (241)	331 (370)*	266 (278)*
1700	3 (171)	362 (332)*	360 (360)*
1800	16 (225)	317 (281)*	333 (343)*
1900	30 (253)	161 (265)	252 (318)
2000	-67 (182)	177 (367)	262 (313)*

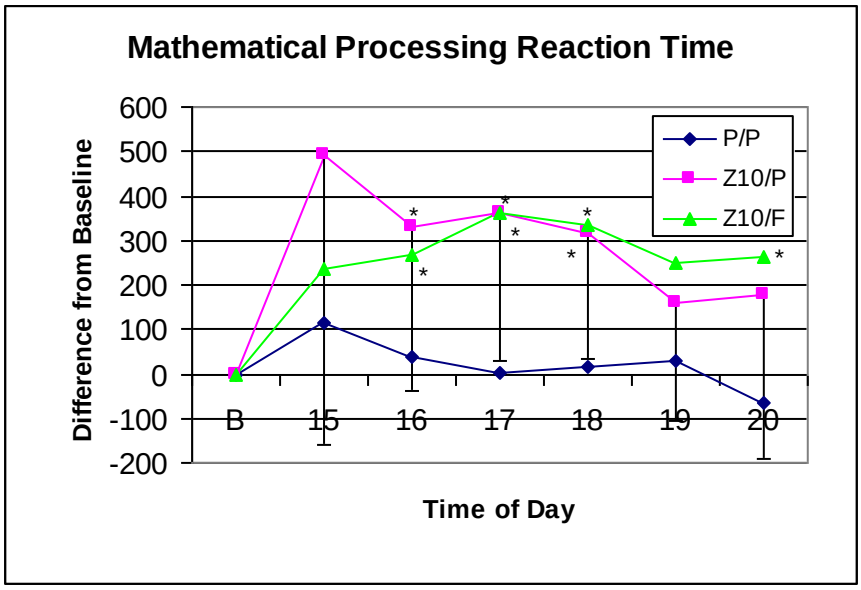

Figure 3. Mathematical processing reaction time as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P; error bars represent the standard error of the mean.)

For mathematical processing throughput, the ANOVA resulted in significant effects for drug and time ($p < 0.05$), but again not for the interaction ($F(4, 37) = 1.608$, $p = 0.196$) using Huynh-Feldt correction. Changes from baseline are shown in Table 8 for each drug condition. The Z10/P change from baseline differed significantly from the P/P condition at 1500 through 1800, whereas the Z10/F change differed significantly from the P/P condition at 1600 through 1800 and also at 2000 ($p < 0.05$). At 2000 there was a trend for Z10/P ($p = 0.059$) and at 1900 a trend for Z10/F ($p = 0.051$). Similar to mathematical processing reaction time and grammatical reasoning throughput, Figure 4 shows that flumazenil appears to only protect performance during the first hour of administration. One hour after flumazenil administration, performance was no better under Z10/F than Z10/P.

Table 8. Change from Baseline in Each Drug Condition for Mathematical Processing Throughput (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	40.29 (11.21)	41.62 (12.23)	41.09 (11.30)
1500	-0.80 (5.29)	-9.61 (10.99)*	-3.83 (5.79)
1600	0.91 (6.04)	-6.24 (7.56)*	-5.08 (4.28)*
1700	1.17 (4.05)	-7.28 (6.24)*	-6.17 (7.21)*
1800	2.09 (4.54)	-8.07 (8.09)*	-6.00 (6.91)*
1900	0.74 (6.45)	-5.47 (8.10)	-4.86 (4.91)
2000	2.01 (5.01)	-4.22 (8.16)	-4.29 (6.90)*
* significantly different from P/P ($p \leq 0.05$)			

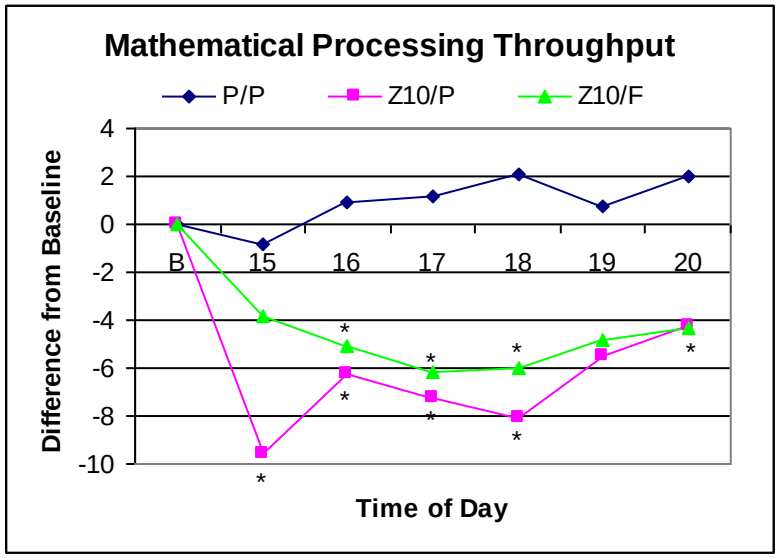

Figure 4. Mathematical processing throughput as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P.)

Continuous Processing

No significant effects were detected by ANOVA for continuous processing task accuracy ($p > 0.05$, see Table 9). The change from baseline for mean reaction time, showed significant effects for drug and time ($p < 0.05$), but not for the drug by time interaction ($F(3, 38) = 1.39$, $p = 0.259$, Huynh-Feldt corrected). As shown in Table 10, significant difference scores were found for Z10/P and Z10/F when compared to P/P at 1500 and 1800 ($p < 0.05$) and a trend was seen at 1600 for Z10/P ($p = 0.054$). Figure 5 shows the degrading performance effects of zolpidem at 1500 and 1600 compared to P/P with reaction time in the Z10/F condition situated between them. The Z10/P and Z10/F effects at 1800 appeared to result from the improved performance of the P/P condition.

Table 9. Change from Baseline in Each Drug Condition for Continuous Processing Task Accuracy (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	98.70 (1.37)	97.89 (1.68)	97.43 (1.37)
1500	-1.52 (1.69)	-8.87 (13.60)	-2.13 (5.51)
1600	-1.19 (2.32)	-4.98 (9.93)	-1.86 (3.67)
1700	-0.98 (1.63)	-9.82 (15.09)	-4.07 (5.18)
1800	-1.93 (3.08)	-7.24 (16.17)	-1.95 (3.69)
1900	-1.89 (3.34)	-8.68 (20.47)	-1.28 (1.77)
2000	-1.53 (3.02)	-4.43 (10.38)	-0.36 (2.55)

Table 10. Change from Baseline in Each Drug Condition for Continuous Processing Task Mean Reaction Time (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	416.6 (65.6)	431.2 (102.8)	430.1 (96.4)
1500	-2.3 (39.1)	74.2 (113.8)*	21.8 (25.0)*
1600	-10.3 (28.8)	33.6 (71.0)	18.7 (32.9)
1700	-2.1 (30.7)	38.9 (78.0)	34.5 (77.7)
1800	-15.5 (43.9)	25.7 (43.9)*	33.0 (74.4)*
1900	-0.4 (64.7)	12.4 (42.4)	6.4 (64.0)
2000	-13.3 (48.0)	8.0 (54.7)	3.7 (56.5)
* significantly different from P/P ($p \leq 0.05$)			

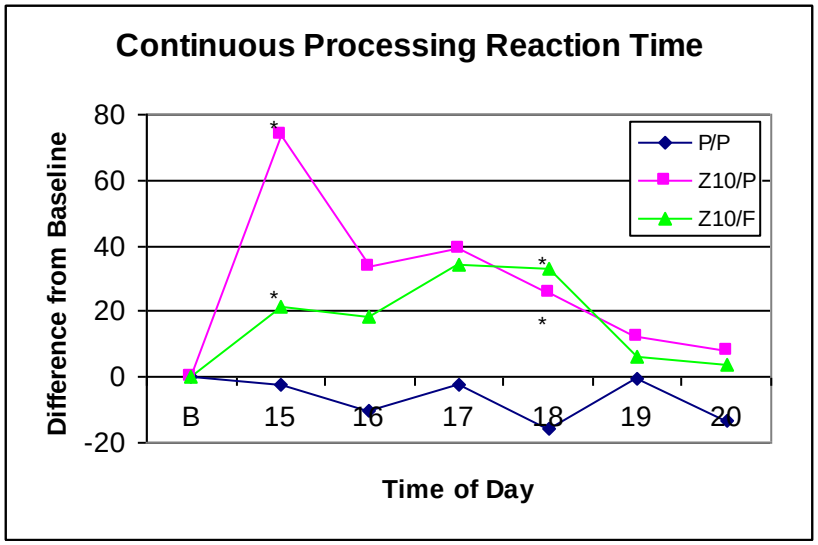

Figure 5. Continuous processing reaction time as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P.)

For continuous processing throughput, the ANOVA resulted in significant effects for drug and time ($p < 0.05$), but again not for the interaction ($F(3, 32) = 1.378$, $p = 0.267$) using Huynh-Feldt correction. Changes from baseline at each hour for each drug condition are shown in Table 11. The Z10/P condition was significantly different from the P/P condition at every hour and it was also significantly different from Z10/F at 1500 ($p < 0.05$). Although the statistical results appear to be very clear that the Z10/P condition was degrading performance while the Z10/F was no different than P/P, Figure 6 shows the Z10/F condition to be intermediate between the two (except at 1500), similar to other cognitive performance dependent measures.

Table 11. Change from Baseline in Each Drug Condition for Continuous Processing Task Throughput (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	145.11 (19.49)	141.72 (27.06)	140.50 (23.78)
1500	-1.81 (10.35)	-30.28 (34.23)* +	-7.15 (11.34)
1600	1.78 (7.79)	-15.17 (26.24)*	-5.55 (12.50)
1700	-1.26 (8.58)	-25.44 (26.20)*	-16.04 (26.61)
1800	1.96 (13.41)	-16.16 (23.65)*	-12.38 (25.19)
1900	-1.18 (19.20)	-15.89 (26.88)*	-5.98 (19.57)
2000	2.15 (14.73)	-10.92 (19.08)*	-2.26 (18.12)

* significantly different from P/P ($p \leq 0.05$)
+ significantly different from Z10/F ($p \leq 0.05$)

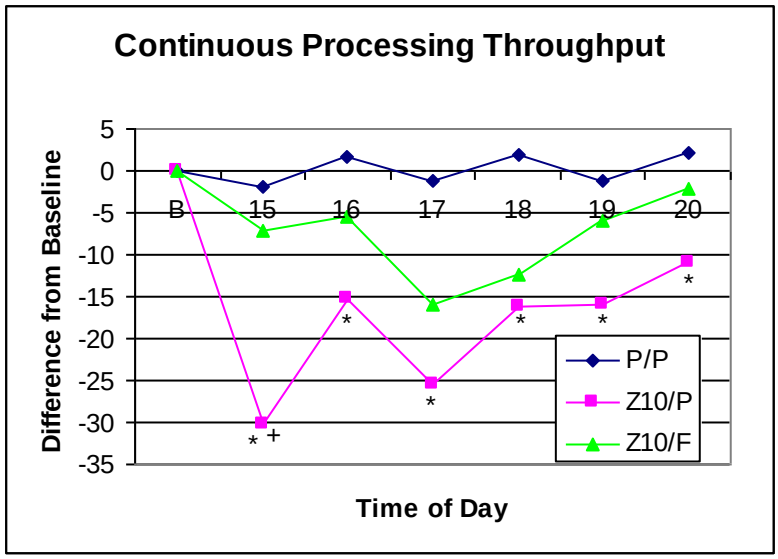

Figure 6. Continuous processing throughput as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P, + indicates significantly different from Z10/F.)

Psychomotor Vigilance Task (PVT) and Simple Reaction Time

The PVT change from baseline measures, mean reaction time and mean reciprocal reaction time, did not show any significant effects when tested by ANOVA. The mean changes from baseline and their standard deviations are shown in Tables 12 and 13. With no significant main effects or interaction, paired comparisons at each hour of testing were not pursued.

Table 12. Change from Baseline in Each Drug Condition for PVT Mean Reaction Time (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	265.0 (37.8)	288.3 (86.8)	280.6 (72.4)
1500	-12.9 (29.7)	281.5 (599.6)	84.7 (175.2)
1600	2.4 (34.1)	253.7 (657.1)	137.9 (334.1)
1700	-4.0 (37.2)	495.5 (1042.3)	27.6 (62.2)
1800	13.7 (62.7)	618.1 (1420.2)	47.9 (74.5)
1900	74.7 (211.4)	416.6 (906.2)	8.0 (78.8)
2000	23.4 (95.8)	295.4 (870.9)	1.9 (58.2)

Table 13. Change from Baseline in Each Drug Condition for PVT Mean Reciprocal Reaction Time (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	4.037 (0.541)	3.859 (0.589)	3.929 (0.476)
1500	0.102 (0.293)	-0.243 (0.599)	-0.304 (0.685)
1600	-0.076 (0.331)	-0.446 (0.677)	-0.437 (0.379)
1700	-0.026 (0.413)	-0.559 (0.657)	-0217 (0.486)
1800	-0.126 (0.589)	-0.509 (0.616)	-0.271 (0.481)
1900	-0.291 (0.633)	-0.508 (0.487)	-0.130 (0.480)
2000	-0.153 (0.563)	-0.261 (0.517)	-0.057 (0.444)

The simple reaction time changes from baseline were not significantly different among the Z10/P, Z10/F, and P/P conditions when tested by ANOVA. Table 14 shows the mean changes from baseline for each drug condition. Since ANOVA results were not significant, no paired comparisons were performed.

Table 14. Change from Baseline in Each Drug Condition for Simple Reaction Time (SD)

Time of Day	P/P	Z10/P	Z10/F
Baseline	213.41 (45.84)	206.62 (24.83)	213.04 (26.21)
1500	6.03 (24.62)	27.16 (38.83)	9.53 (20.27)
1600	-9.10 (24.55)	16.35 (29.89)	8.24 (27.14)
1700	-0.70 (22.51)	42.51 (90.82)	4.51 (27.34)
1800	-5.50 (28.13)	61.34 (145.43)	12.81 (31.68)
1900	1.53 (32.71)	29.30 (88.63)	5.38 (28.62)
2000	-4.64 (29.53)	4.50 (22.22)	-9.25 (28.87)

Memory

The Williams Word memory test was administered after the first awakening from sleep (1500) and again at 1700. For the number of words correctly recalled, a repeated-measures

analysis of variance with two within-subject factors, drug condition (the three drug combinations) and time (1500 and 1700), was performed to test for a significant drug and time main effect and a drug by time interaction. The ANOVA resulted in significant effects for time ($F(1, 11) = 44.759$, $p = 0.001$ using Huynh-Feldt correction), but not for drug ($F(1, 15) = 2.904$, $p = 0.100$ using Huynh-Feldt correction), or the interaction ($F(2, 32) = 1.900$, $p = 0.173$). The number of correctly recalled words and the standard deviation at each hour are shown in Table 15. The Z10/P condition was significantly different from the P/P condition at 1500 ($p = 0.021$). No other pair wise comparisons were found to be significant. Figure 7 shows the mean number of recalled items at each time for each drug condition.

Table 15. Mean Words Recalled by Drug Condition (SD)

Time of Day	P/P	Z10/P	Z10/F
1500	12.08 (1.68)	8.75 (4.16)*	10.92 (3.55)
1700	8.17 (3.10)	6.42 (4.08)	8.75 (3.62)
* significantly different from P/P ($p \leq 0.05$)			

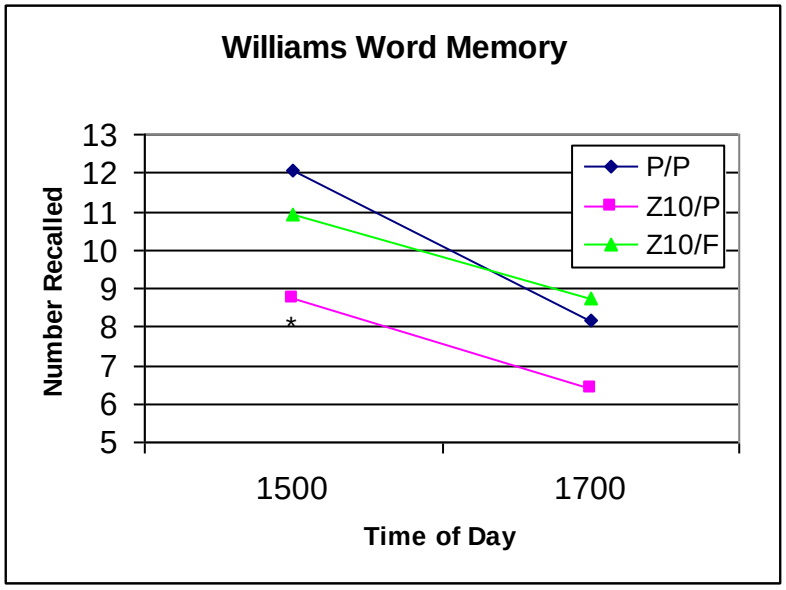

Figure 7. Williams Word Memory, number correct as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P.)

PHYSIOLOGICAL PERFORMANCE

Two measures of physiological state were used to assess the vestibular system and physical strength. Both postural sway and grip strength are interval measures and were subjected to the same statistical analysis as the cognitive performance measures. Unfortunately, the postural sway data were incomplete and the data available appeared to be corrupt. Therefore, these data are not presented.

Grip Strength

Physical strength was measured with a hydraulic hand dynamometer. None of the factors individually (drug or time) or in combination (drug by time interaction, ($F(4, 35) = 1.621$, $p = 0.192$)) had an effect on grip strength. However the values at 1500 presented the same pattern as many of the cognitive variables with the Z10/F falling between the P/P and the Z10/P conditions.

SUBJECTIVE REPORT

Sleepiness

The Stanford Sleepiness Scale (SSS) provided numerical ratings of subjective sleepiness. These were analyzed with ANOVA using the same procedures as with the cognitive test data ($\alpha = 0.05$). There were significant effects for time ($p < 0.05$) and for the drug by time interaction ($F(12, 72) = 2.22$, $p = 0.019$). As shown in Table 16, subjective sleepiness was significantly higher for Z10/P than for P/P and Z10/F at 1600. At 2000, subjective sleepiness was statistically higher in the P/P condition than in the Z10/F condition. Figure 8 shows the degrading performance effects of zolpidem at 1600 compared to P/P and Z10/F. The high sleepiness rating in the P/P condition at 2000 is somewhat explained by one participant giving a high rating compared to the rest of the participants; Table 16 shows the standard deviation to be the highest for any condition at any time. Interestingly, the SSS showed a significant elevation from baseline at 1500 under P/P reflecting the effect of sleep inertia. Similarly, the Z10/P condition showed differences from its baseline at 1500 and 1600 ($p < 0.05$).

Table 16. Change from Baseline in Each Drug Condition for the Stanford Sleepiness Scale (SD).

Time of Day	P/P	Z10/P	Z10/F
Baseline	1.57 (0.54)	1.71 (0.49)	2.14 (0.90)
1500	0.86 (0.69)	1.57 (1.13)	0.57 (0.98)
1600	0.29 (0.95)	1.14 (1.22)* $^{+}$	0.14 (1.07)
1700	0.43 (1.13)	0.86 (1.07)	0.29 (1.25)
1800	0.57 (.98)	0.86 (1.07)	0.43 (1.13)
1900	0.71 (1.25)	0.71 (0.95)	0.29 (0.76)
2000	1.14 (1.46)	0.14 (0.69)	0.00 (0.82)*

* significantly different from P/P ($p \leq 0.05$), n = 7
$^{+}$ significantly different from Z10/F ($p \leq 0.05$)

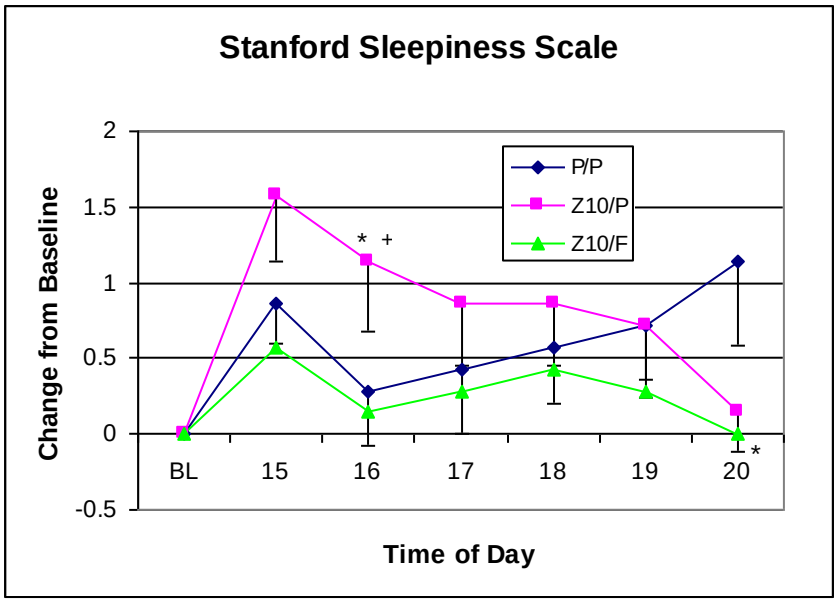

Figure 8. Stanford Sleepiness Scale as affected by the three drug conditions: P/P, Z10/P, and Z10/F. (* indicates significantly different from P/P, $^{+}$ indicates significantly different from Z10/F; error bars represent the standard error of the mean.)

Symptoms

Participants completed a 73-item paper and pencil Symptom Checklist at the end of each test block, indicating the severity (none, some, moderate, or severe) they were experiencing for each symptom at that point in time. Only symptoms showing an increase from baseline were examined for drug effects with the Wilcoxon Signed Rank test. Further, only those symptoms for which at least 25% of the participants (i.e., at least 3 participants) exhibited an increased severity under at least one of the conditions are shown in Table 17. The tabled value is the percentage of participants showing an increase from baseline for the symptom at the time of testing. Bolded values represent conditions that are significantly different from the P/P condition ($p < 0.05$).

From the analysis, a significant number of participants presented symptoms of Trouble Staying Awake, "Drugged" Feeling, Light headed, and Difficulty Concentrating. Participants experienced these symptoms between one and three hours after zolpidem administration (1500-1700) and six of the eight increases were under the Z10/P condition. For the Z10/F condition, the "Drugged" Feeling and Light Headed symptoms were significant at 1500 and 1700, respectively.

Table 17. Symptoms Showing Increased Severity by Time and Drug Condition.

Symptom	Condition	1500	1600	1700	1800	1900	2000
Trouble Staying Awake	P/P	0.0	0.0	0.0	8.3	16.7	8.3
	Z10/P	27.3	18.2	**36.4**	30.0	36.4	18.2
	Z10/F	8.3	16.7	25.0	25.0	25.0	16.7
"Drugged" Feeling	P/P	0.0	0.0	0.0	0.0	0.0	0.0
	Z10/P	36.4	**45.5**	**54.5**	20.0	18.2	9.1
	Z10/F	**33.3**	41.7	33.3	33.3	8.3	16.7
Light headed	P/P	8.3	0.0	0.0	0.0	0.0	0.0
	Z10/P	27.3	**36.4**	27.3	20.0	18.2	9.1
	Z10/F	25.0	25.0	**41.7**	16.7	0.0	0.0
Loss of Balance	P/P	0.0	0.0	0.0	0.0	0.0	0.0
	Z10/P	9.1	9.1	27.3	20.0	9.1	9.1
	Z10/F	16.7	25.0	8.3	8.3	0.0	0.0
Fatigue	P/P	8.3	8.3	8.3	8.3	16.7	25.0
	Z10/P	18.2	18.2	18.2	30.0	18.2	9.1
	Z10/F	8.3	8.3	16.7	8.3	8.3	8.3
Drowsiness	P/P	8.3	0.0	8.3	8.3	25.0	16.7
	Z10/P	36.4	27.3	45.5	20.0	36.4	27.3
	Z10/F	16.7	8.3	16.7	25.0	8.3	8.3
Headache	P/P	16.7	16.7	8.3	8.3	8.3	8.3
	Z10/P	9.1	18.2	18.2	10.0	9.1	18.2
	Z10/F	16.7	25.0	8.3	16.7	8.3	8.3
Difficulty Focusing	P/P	0.0	8.3	0.0	0.0	0.0	0.0
	Z10/P	36.4	18.2	36.4	30.0	27.3	18.2
	Z10/F	8.3	25.0	8.3	0.0	8.3	0.0
Nausea	P/P	0.0	16.7	0.0	0.0	0.0	0.0
	Z10/P	18.2	27.3	27.3	20.0	9.1	9.1
	Z10/F	16.7	25.0	16.7	25.0	8.3	8.3
Difficulty Concentrating	P/P	0.0	0.0	0.0	0.0	0.0	0.0
	Z10/P	**27.3**	27.3	**45.5**	30.0	18.2	18.2
	Z10/F	16.7	8.3	16.7	0.0	0.0	0.0
Stomach Awareness	P/P	16.7	25.0	0.0	8.3	0.0	0.0
	Z10/P	9.1	9.1	9.1	10.0	9.1	9.1
	Z10/F	8.3	16.7	8.3	8.3	8.3	8.3
Vivid Dreams	P/P	0.0	0.0	0.0	0.0	0.0	0.0
	Z10/P	9.1	0.0	0.0	0.0	0.0	0.0
	Z10/F	25.0	8.3	0.0	0.0	0.0	0.0

Note: Bold values were significantly higher than P/P, $p \leq 0.05$, using the Wilcoxon Signed Rank test.

Examining the subjective symptoms reported by participants and collapsing across the various sample times, we were able identify symptoms associated with each drug condition. Table 18 shows the percentage of participants reporting symptoms at a level higher than baseline

regardless of the time. Bolded values represent significantly increased symptom severity compared with the P/P condition ($p < 0.05$).

Table 18. Percentage of Participants Reporting Symptoms within Each Drug Condition at Any Time.

Symptom	P/P	Z10/P	Z10/F
Trouble Staying Awake	16.7	**54.5**	25.0
Visual Illusions	0.0	9.1	25.0
"Drugged" Feeling	0.0	**63.6**	**83.3**
Light headed	8.3	**45.5**	**50.0**
Difficulty Staying Awake	8.3	36.4	25.0
Loss of Balance	0.0	27.3	**50.0**
Loss of Coordination	0.0	27.3	25.0
Fatigue	25.0	45.5	33.3
Drowsiness	25.0	63.6	41.7
Headache	16.7	36.4	25.0
Eye Strain	8.3	27.3	25.0
Difficulty Focusing	8.3	**54.5**	41.7
Nausea	16.7	27.3	33.3
Difficulty Concentrating	0.0	**54.5**	**41.7**

Note: Bold values were significantly different from P/P, $p \leq 0.05$, using the Wilcoxon Signed Rank test.

Table 18 shows that participants experienced many symptoms under the Z10/P and Z10/F conditions, five and four respectively. Whereas participants under the Z10/P condition appeared to experience the "Drugged" Feeling more frequently than the Z10/F condition (Table 17), when only the number of participants are considered collapsing across time, the percentage for the Z10/F condition was 83.3 percent (10 out of 12) exceeding that of Z10/P (63.6). Similarly for Loss of Balance, half the participants experienced an increase in this symptom at some time whereas no single time was significant. The Z10/P condition showed a similar effect for Difficulty Focusing including 54.5 percent of the participants. Again for Difficulty Concentrating, the Z10/F condition increased this symptom to a significant 41.7 percent. Somewhat surprisingly participants did not identify either Fatigue or Drowsiness as increasing under any of the conditions. However, 25 percent of participants indicated at baseline that they experienced these symptoms, thus setting a high mark to overcome with only a four point scale.

Subjective evaluations of mood were acquired using the ANAM Mood Scale II. Unfortunately, these data were lost during a move from one building to another.

DISCUSSION

The overall results of this investigation demonstrate that the sublingual administration of flumazenil can at least partially nullify the soporific effects of zolpidem. These findings confirm those of Wesensten et al. (1995) and others who found impairment reversed by intravenous administration of flumazenil, but that the effect of sedation returned within six hours of the original zolpidem administration. While the debilitating effects of zolpidem were shown on cognitive performance, memory, sleepiness, and side effects, sublingual flumazenil only reversed these effects for one to two hours. While these effects can not be seen clearly in the reaction time measure of the three cognitive tests, Figure 9 shows this effect for the throughput measure. It shows the number of significant differences when comparing Z10/P and Z10/F with P/P at each time. Performance under Z10/F typically fell between Z10/P and P/P for the first hour or two and then often joined the Z10/P performance curve as performance returned to that of the P/P condition with the metabolism of the zolpidem. Although accuracy frequently does not show significant effects because of its limited range, the Z10/P condition showed 8-10 percent degradation relative to P/P while the Z10/F conditions showed 2-3 percent degradation. The drug conditions also affected the Williams Word Memory test similar to the other measures. Participants in the flumazenil condition recalled nearly as many words as the P/P condition while in the zolopidem-only condition participants recalled approximately three fewer words.

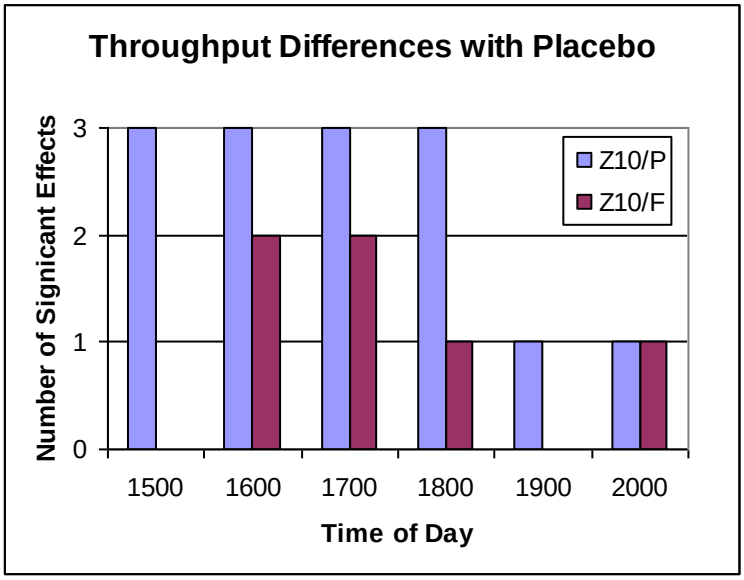

Figure 9. This figure summarizes the results of the three cognitive tests by showing the number of significant differences with P/P at each time.

While our only physiological measure (grip strength) for Z10/P was not different from P/P, the subjective measures of sleepiness and symptoms showed significant effects for Z10/P. Interestingly, the Stanford Sleepiness Scale showed that flumazenil almost completely nullified the sleepiness effects of zolpidem for the duration of the data collection session. However, the high baseline level of sleepiness for the zolpidem plus flumazenil condition contributed to this

effect by reducing the differences for the subsequent time samples. The symptom results present a picture of flumazenil only partially nullifying the effects of zolpidem. Under Z10/P, a significant number of participants indicated they had "trouble staying awake," felt "drugged," felt "light headed," and had "difficulty concentrating" from one to three hours after zolpidem administration. Flumazenil helped to eliminate most of these symptoms, but still left most participants experiencing some of these symptoms at some time during the data collection period.

One other way of understanding these data is to look at the restorative value of flumazenil on the percentage of degradation induced by zolpidem. Using the throughput measures for each of the three cognitive tests, the sample means for each time and drug condition were divided by the condition's baseline. Then each proportion was divided by the P/P value for each time and multiplied by 100. These percentages for each test were used to compute a mean for Z10/P and Z10/F at each time. Figure 10 shows a plot of these values, average percent change from P/P. From this chart it can be seen that zolpidem degrades performance about 25%, 90 minutes after administration. After flumazenil administration, these data show that performance is restored to 92%, a significant 17% improvement. An hour later after the second administration of flumazenil, performance drops another 5% providing only a 4% improvement over Z10/P. In the next hour, performance in the flumazenil condition drops another 6% to 82%, providing only a 5% improvement over Z10/P. Thereafter, zolpidem is assumed to slowly metabolize allowing performance to recover. However, even at 2000, performance remains 10-11% degraded compared to P/P. In the Williams Word Memory test, similar percentages were found at 1500, but at 1700 flumazenil appeared to completely restore memory to the same level as P/P. The Z10/P condition was restored to 90% of the P/P condition at 1700.

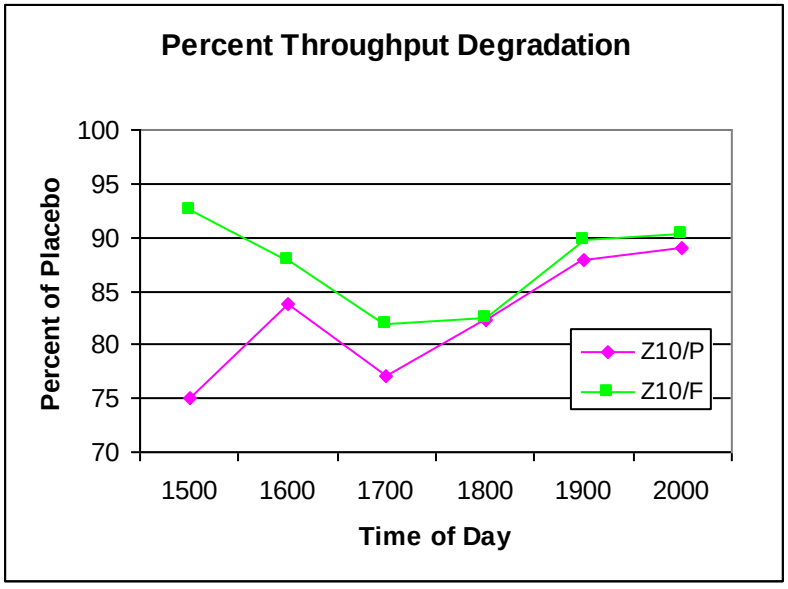

Figure 10. Percent degradation from P/P across throughput measures for three cognitive tests. Zolpidem administered at 1330.

The failure of the second administration of flumazenil to restore performance at 1600 is at present unexplained. One hypothesis is that flumazenil may compete with zolpidem for receptor sites within the liver. The zolpidem displaced from ω_1 and CYP 3A4 sites could remain in the serum continuing to promote sedation. This is only seen when the quantities of both drugs are sufficient to saturate the liver CYP 3A4 enzyme binding. In this study, the failure of flumazenil to continue the restoration of performance after the second dose lends weak support to the finding of Bonfiglio, Fisher-Katz, Saltis, et al. (1996) that found 1 mg of intravenous flumazenil prolonged the elimination half-life of midazolam. While their study was based on only four participants and midazolam has a different elimination rate than zolpidem, this is a hypothesis worth testing in future research.

ZOLPIDEM SIDE EFFECTS

A fourth arm of this study, administering 20 mg of zolpidem, then two doses of sublingual flumazenil, was discontinued after three of the exposed four participants experienced vomiting, two with projectile vomiting. The fourth participant experienced only nausea. This incidence of nausea and vomiting is higher than seen in a previous study using 20 mg of zolpidem without flumazenil administration. Conversation with the lead author of the study that showed flumazenil to reverse memory impairment (reference 12) due to zolpidem revealed their incidence of nausea and vomiting to be around 60 % with 20 mg of zolpidem. Though 20 mg of zolpidem is greater than the FDA approved doses of 5 and 10 mg, clinical trials with 20 mg doses report an incidence of approximately 2% for nausea. There is a possibility that interaction between high doses of flumazenil and zolpidem results in nausea and vomiting.

FLUMAZENIL AND OTHER HYPNOTICS

Currently, temazepam, zolpidem, and zaleplon are the only hypnotic agents approved for use by USAF aircrew. A PubMed search did not find any studies on using flumazenil to reverse sedation due to temazepam. Publications were found related to studies administering flumazenil to precipitate withdrawal symptoms with zaleplon use but not specifically to reverse sedation. Zopiclone is another non-benzodiazepine hypnotic that offers some benefits compared to the currently USAF approved hypnotic medications. It has a half-life similar to temazepam, making it suitable for use to reduce fatigue due to circadian rhythm disruption. Unlike temazepam, it maintains a normal proportion of slow-wave sleep during an eight-hour sleep period. It has been well studied for 25 years and is available in Europe and Canada. The s-enantimer, eszopiclone, recently became available in the United States under the brand name Lunesta. Zopiclone does not bind directly to the ω_1 and ω_2 subunits but to a related, allosteric site. An in vitro experiment demonstrated this when a single dose of flumazenil fully reversed all zopiclone influence at the GABA receptor. This was not true for zolpidem, triazolam, and flunitrazepam. The combination of eszopiclone and sublingual flumazenil suggests the possibility of inducing normal sleep architecture of any desired duration up to eight hours and awakening quickly after flumazenil administration without risk of resedation.

CONCLUSIONS

Under the conditions of this experiment, the data and analysis provide the following conclusions.

1. At the peak of the serum saturation, 10 mg zolpidem degrades cognitive function to approximately 75% of a placebo control condition.
2. Sublingual flumazenil, administered immediately on awakening, was shown to reverse the cognitively degrading effects by 23%, restoring performance to 92.5% of the placebo control condition.
3. A second administration of flumazenil one hour post awakening had no beneficial effects.
4. One to two hours after awakening, performance did not return to the level of the placebo control condition after flumazenil administration, but rather joined the zolpidem decay function which continued to be approximately 20% degraded compared to the placebo control condition.
5. At five hours post awakening, performance remained degraded by 10-11% compared to the placebo control condition.

The improvement in the throughput measure for three cognitive performance tests demonstrates that further research using more sophisticated formulations should be continued. Flumazenil is likely to show greater antagonism of other hypnotic medications, such as eszopiclone, and provide more complete restoration of performance over a longer period of time.

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